HHS Public Access

Author manuscript

Cell Calcium. Author manuscript; available in PMC 2017 March 01.

Published in final edited form as:

Cell Calcium. 2016 March ; 59(2-3): 98–107. doi:10.1016/j.ceca.2015.12.002.

Calcium signaling in human stem cell-derived cardiomyocytes: Evidence from normal subjects and CPVT afflicted patients

Xiao-Hua Zhang and **Martin Morad**

Cardiac Signaling Center of USC, MUSC, & Clemson University, Charleston, SC 29425

Abstract

Derivation of cardiomyocyte cell lines from human fibroblasts (induced pluripotent stem cells, iPSCs) has made it possible not only to investigate the electrophysiological and Ca^{2+} signaling properties of these cells, but also to determine the altered electrophysiological and Ca^{2+}-signaling profiles of such cells lines derived from patients expressing mutation-inducing pathologies. This approach has the potential of generating *in vitro* human models of cardiovascular diseases where cellular pathology can be investigated in detail and possibly specific pharmacotherapy developed. Although this approach has been applied to a number of mutations in channel proteins that cause arrhythmias, there are only few detailed reports addressing Ca^{2+} signaling pathologies beyond measurements of Ca^{2+} transients in intact non-voltage clamped cells. Unfortunately full understanding of Ca^{2+} signaling pathologies remains elusive, not only because of the plethora of Ca^{2+} signaling proteins defects that cause arrhythmias and cardiomyopathies, but also because detailed functional properties of Ca^{2+} signaling proteins are difficult to obtain. Catecholaminergic polymorphic ventricular tachycardia (CPVT1) is a malignant inherited arrhythmogenic disorder predominantly caused by mutations in the cardiac ryanodine receptor (RyR2). Thus far over 150 mutations in RyR2 have been identified that appear to cause this arrhythmia, a number of which have been expressed and studied in transgenic mice or cell-line models. The development of human iPSC-technology makes it possible to create human heart cell-lines carrying these mutations, making detailed identification of Ca^{2+} signaling defects and its specific pharmacotherapy possible.

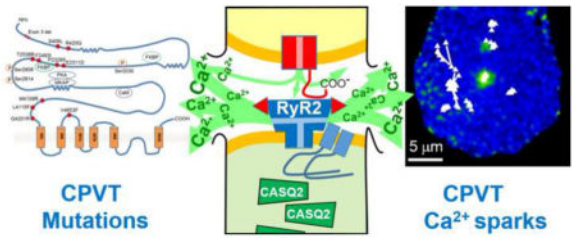
In this review we shall first briefly summarize the essential characteristics of the mammalian cardiac Ca^{2+} signaling, then compare them to Ca^{2+} signaling phenotypes of human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CM) and to those of rat neonatal cardiomyocytes, and categorize the possible variance in Ca^{2+} signaling defects caused by different CPVT-inducing mutations as expressed in hiPSC-CMs.

Graphical Abstract

Corresponding Author: Martin Morad, Cardiac Signaling Center of University of South Carolina, Medical University of South Carolina and Clemson University, MUSC, 68 President St, BEB 306 Charleston, SC 29425 USA, moradm@musc.edu, Office: +1 843-876-2400, Fax: +1 843-876-2489, Mobile: +1 803-873-7113.

Conflict of interest

None of the authors have any actual or potential conflict of interest.



Keywords

Catecholaminergic polymorphic ventricular tachycardia (CPVT); cardiomyocytes derived from human induced pluripotent stem cells (hiPSC-CM); Ca^{2+} signaling; stem-cell-derived disease models; ryanodine receptor

1. Calcium Signaling in adult Cardiomyocytes

Following seminal experiments of Ringer some 150 years ago it became apparent that Ca^{2+} played a central role in initiation and regulation of cardiac contractility, but It was not until the 1960s that the advent of technologies of voltage-clamp for ventricular strips [1, 2], the development of Ca^{2+} sensitive dyes and the whole-cell patch-clamp technique for single cells [3,4] made it possible to explore the intricate details of cardiac Ca^{2+} signaling. The picture that emerged suggested that the entry of Ca^{2+} through the Ca^{2+} channel, during the action potential, triggers much larger releases of Ca^{2+} from the RyR2 receptors [5] located on the dyadic junctions of sarcoplasmic reticulum (SR), setting in motion the activation of contraction (Fig. 1). This process became known as Ca^{2+}-induced Ca^{2+}-release (CICR, [6]), a mechanism that seems to have evolved specifically in mammalian heart versus a faster voltage-gated Ca^{2+} release (VICR) mechanism in skeletal muscle. The advent confocal Ca^{2+} imaging in line-scan mode made it possible to capture focal rise of Ca^{2+} in the dyadic micro-domains between SR and t-tubular membranes, known as Ca^{2+} spark [7]. Ca^{2+} spark are sporadic events that occur during diastole or are triggered by activation of I_{Ca}. Cleemann *et al.* [8] using 2-D rapid (240 f/s) confocal Ca^{2+} imaging in whole cell clamped ventricular myocytes dialyzed with high concentrations of EGTA to limit the diffusion of Ca^{2+}-bound Fluo-3, imaged simultaneously over 100s of sparks and found spark durations of ~7ms, and spark amplitudes that were invariable with voltage, but spark frequencies that were dependent on the magnitude of I_{Ca}. These studies also showed that a Ca^{2+} spark represented the release of ~100,000 calcium ions, resulting most likely from activation of ryanodine receptor clusters in a dyadic junction. Consistent with this idea the sparks occurred at the same cellular location with over 70% consistency from beat to beat (8).

It is important to note that although CICR is a ligand-gated process, it has significant voltage-dependence [9], such that the gain (amplification factor) of the process may exceed 100 at −40 to −30 mVs, but it drops to values below 15 at voltages positive to 10 mVs, [10]. The mechanism for this unexpected property of CICR remains somewhat controversial, but experiments on rat atrial myocytes using rapid 2-D (240 f/s) confocal imaging have identified two groups of RyR2: those near the surface membrane, associated with dihydropyridine receptors (DHPRs), showing significant voltage-dependence and those

centrally located, not associated with DHPRs, showing no voltage-dependence [11]. These findings imply that the voltage-dependence of gain of CICR reflects possible direct interaction of RyR2 with Ca^{2+} channels. In support of this possibility, cellular introduction of LA-fragments of cloned carboxyl-tail of alpha subunit of Ca^{2+} channel into freshly isolated rat atrial myocytes imparted voltage-dependence to the CICR of centrally located "naked" RyR2. This finding was specific to LA, but not to the K fragment of carboxyl-tail that includes the I-Q motif [11].

Calcium signaling during evolution appears to have evolved from a surface-membrane (Ca^{2+}-channel/ NCX) dominated process, as in amphibian & shark hearts [2, 12], to an intracellular regulated system, in mammalian hearts, where the Ca^{2+} channel serves as a trigger to release of Ca^{2+} from an internal Ca^{2+} stores of SR, and where Ca^{2+} is sequestered via SERCA2a/Phospholamban proteins and stored on Ca^{2+}-binding protein calsequestrin [13]. In mammalian hearts with morphological changes that accompany growth and differentiation of the heart that includes the development of t-tubules, SR, adrenergic receptors, and myofilaments, there appears also to be a shift from Ca^{2+} entry across the membrane to Ca^{2+} release from intracellular stores.

In addition to the fast I_{Ca}-gated CICR mechanism, a slower IP_3-gated Ca^{2+} release mechanism appears also to have evolved in the mammalian hearts [14]. This signaling pathway appears to be more dominant in immature developing heart, possibly playing a critical role in nuclear signaling during growth and development [15–18]. The role of IP_3-gated signaling in EC-coupling of healthy adult hearts remains somewhat cloudy, but significant data supports over expression of IP_3-gated signaling pathway in a number of cardiac pathologies [19–21].

Mitochondria also appear to contribute to Ca^{2+} signaling processes of cardiac myocytes. Early studies on isolated mitochondria show that mitochondria sequester and release Ca^{2+} from and to the cytosol [22]. The sequestered Ca^{2+} is thought to stimulate production of nitric oxide (NO), which in turn regulates oxygen consumption, ATP production, and ROS generation [23]. Nevertheless, it remains still unclear whether mitochondria play a critical role in beat-to-beat Ca^{2+} regulation of the myocyte. Since mitochondria make up some 30–40% of the cardiac cell volume, their contribution to cellular Ca^{2+} signaling could be significant [24]. In support of direct role for mitochondria in cellular Ca^{2+} signaling, shear stress pulses (pressurized flow of solution) appeared to trigger Ca^{2+} transients that originate from mitochondrial Ca^{2+} pools, by a mechanism independent of CICR, that had mitochondrial pharmacology [25]. The notion of a rapid (~500 ms) mitochondrial Ca^{2+} release was also confirmed in different cardiac cell types (adult ventricular cells, rat neonatal cardiomyocytes and hiPS-CM), under voltage-clamp conditions, and with genetically targeted mitochondrial probes. This is somewhat surprising, but is supported by key observations that: a) shear stress pulses induce Ca^{2+} release only after Ca^{2+}-loading; b) perinuclear and peripheral mitochondrial populations often produce simultaneous Ca^{2+} signals with opposite polarity (uptake vs. release); c) the propensity of mitochondria to release Ca^{2+} increases with Ca^{2+} loading (e.g. by Na^+ withdrawal); and d) mitochondrial Ca^{2+} release may escape detection in whole-cell measurements, where mitochondrial Ca^{2+}

release maybe counter-balanced by uptake signals in different populations of mitochondria [25, 26].

Cardiac Ca^{2+} signaling remains an active area of research as state-of-the-art technologies ranging from molecular, genetic, electrophysiological and imaging approaches become available. Nevertheless, though I_{Ca}-gated Ca^{2+} release is the dominant pathway for activation of contraction in the heart, other pathways that include transport of Ca^{2+} across the membrane or released from ER and mitochondria may critically modulate cardiac Ca^{2+} signaling depending on pathophysiological or developmental stage of the heart.

2. Ca^{2+} signaling in human fibroblast-derived cardiomyocytes (hiPSC-CM)

It is well documented that iPSC-CMs beating clusters display mixed ventricular, atrial, and pacemaker-like cell populations based on their action potential phenotypes [27–29]. Although research is still ongoing to develop protocols that generate purer populations of a specific type of myocyte, the effort to generate homogenous cell populations of atrial, ventricular or pacemaker myocytes, remains to be fully achieved. Characterization of iPSC-CMs has revealed that while molecular, electrophysiological, metabolic, and Ca^{2+} signaling properties of hiPSC-CM are remarkably similar to those of adult cardiomyocytes, the genetic expression pattern, the cellular morphology of hiPSC-CM, might be more similar to the neonatal instead of adult cardiac myocytes [30, 31]. Studies with human embryonic stem cell-derived cardiomyocytes have revealed that t-tubules are absent in these developing cells [31, 32]. Table 1 compares the molecular and functional properties of a number of hiPSC derived cardiomyocyte cell-lines reported thus far. A cursory survey of the reported data, suggests significant differences between different cell lines, even though detailed analysis of Ca^{2+} signaling parameters that includes: the level of expression of Ca^{2+} channels, I_{Ca}-gated Ca release and its gain from RyR2s of SR, the magnitude of SR Ca^{2+} store, and the level of functional expression of NCX and SERCA/PLB and their regulation by adrenergic agonists, or the role of mitochondria in hiPSC-CM Ca^{2+} signaling; is often missing.

2.1. L-type Ca^{2+} current expression and whole cell Ca^{2+} transients

L-type Ca^{2+} channels are critical in excitation-contraction coupling of both cardiac and skeletal muscles. Human or mouse iPSC-CMs express robust levels of L-type Ca^{2+} channels, the gating properties and pharmacology of which are similar to those reported for mammalian and human cardiomyocytes [33–35]. I_{Ca} is activated at potentials positive to −40 mV, reached its peak values of ~ 8 pA/pF at 0 mV, reversed direction becoming outward at potentials positive to +60 mVs (e.g. fig. 2A), and could be blocked by conventional L-type Ca^{2+} channel blockers. The developed Ca^{2+} transients [36, 37] had similar bell-shaped voltage-dependencies as that found for I_{Ca} ; i.e. I_{Ca}-gated Ca^{2+} release activated at −30 mV, peaked at ~ 0 mV, and triggered little or no release of Ca^{2+} at potentials positive to +60 mVs (Fig. 2A). Repolarization from potentials positive to 60 mVs back to the resting potentials also triggered significant release of Ca^{2+} consistent with the idea that the influx of Ca^{2+} and not the depolarization of membrane triggers the release of Ca^{2+}, as proposed for the adult mammalian cardiomyocytes (Fig. 2A, red traces, [38]. Positive depolarizing potentials also activated significant influx of transmembrane Ca^{2+} on the Na^{+}/Ca^{2+} exchanger (NCX),

which appears to contribute significantly to a slower developing component of Ca^{2+} transients [33]. This possibility is supported by the finding that 1 μM nifedipine while completely blocking I_{Ca}, suppresses only the rapidly developing component of Ca^{2+} transients, suggestive of considerable contribution of Na^{+}/Ca^{2+} exchanger to Ca^{2+} transients [33]. This property of hiPSC-CM is consistent with previous findings in developing and neonatal mammalian cardiomyocytes suggestive of a more significant role for Na^{+}/Ca^{2+} exchanger in EC-coupling of developing cardiomyocytes [39]. It should be noted that other groups have reported that in intact, non-patch clamped single or small monolayer clusters of hiPSC-CMs, 1 μM nifedipine completely suppressed the cellular Ca^{2+} transients [40], suggestive of full dependence of Ca^{2+}-transients on I_{Ca}. This finding is not necessarily contradictory to reports in whole cell clamped hiPSC-CM identifying both nifedipine-sensitive and insensitive components Ca^{2+} transients, as in intact cells blocking I_{Ca} would either suppress excitability fully or shorten the action potential to levels that could not support the longer depolarization periods required for the influx of Ca^{2+} on Na^{+}/Ca^{2+} exchanger. Simultaneous recordings of Ca^{2+} transients and contractions from small clusters of iPS-CMs also show similar characteristic as those of adult mammalian cardiomyocytes [41]. Comparison of the Ca^{2+} handling of field-stimulated (0.5Hz) hiPSC-CMs to those of acutely-isolated adult primary ventricular rabbit and mouse cardiomyocytes under identical experimental conditions, also showed that their Ca^{2+} transient amplitudes were not significantly different than those of adult rabbit and mouse cardiomyocytes, whereas time to peak and decay rates of Ca^{2+} transients were significantly slower in hiPSC-CM [34].

The quintessential property of the gain of CICR in cardiomyocytes is its voltage-dependence, (See above, [2, 12]). This characteristic of CICR appears also to be preserved in hiPSC-CM, suggesting that iPSC-derived cardiomyocytes have the phenotypic cardiac Ca^{2+} signaling characteristics, in sharp contrast to the neuronal CICR that shows no indication of voltage-dependence [42]. Thus, the available data on characterization of spontaneously- or depolarization-triggered Ca^{2+} transients suggests that hiPS-CMs express the cardiac-type excitation–contraction-coupling that closely resembles that found in the mammalian myocardium.

2.2. Functional SR Ca^{2+} store: SERCA AND NCX

Molecular studies show that Ca^{2+}-handling proteins of the mammalian heart: RyR2, IP_3R2, SERCA2a, PLN are all expressed in hiPSC-CM [28, 40, 43]. Immuno-fluorescence studies suggest punctuates clustering of RyR2s near the surface membrane in hiPSC-CM (Fig. 2E), consistent with functional findings that Ca^{2+} influx via the L-type Ca^{2+} channels triggers the release of the SR Ca^{2+} stores [33]. Immuno-cyto-staining studies of RyR2 and sarcomeric alpha-actinin, in small mono-layered clusters of hiPSC-CM, show that sarcomeric alpha-actinin staining has a relatively disorganized striated sarcomeric pattern [44]. In another study, RyR2 expression was also detected throughout the cytosol with some myofilaments co-localization [40].

The magnitude SR Ca^{2+} stores of hiPSC-CM has been quantified using rapid and short applications of caffeine pulses. Caffeine concentrations of 3–5 mM triggered large global Ca^{2+} transients that activated inward I_{NCX}, of about 2.5 pA/pF (Fig. 2D) as compared to

~1.0 pA/pF in rat neonatal cardiomyocytes, confirming functionally the existence of large SR Ca^{2+} stores in hiPSC-CM [45]. The larger density of I_{NCX} activated on application of caffeine as compared to values of ~1.0 pA/pF in adult cardiomyocytes of rat, rabbit, mouse, and human hearts suggests either larger stores of Ca^{2+} accessed by caffeine in hiPSC-CM or a greater density of NCX in hiPSC-derived myocytes. Comparison of caffeine triggered Ca^{2+} transients in hiPSC-CM with adult rabbit and mouse cardiomyocytes, in another study [34], suggests equivalent magnitudes, but diverse I_{NCX} values of only 0.6 pA/pF in hiPSC-CM and 1.3pA/pF in rabbit myocytes. This diversity between the two sets of data may have resulted from either higher Ca^{2+} buffering concentrations, or developmental maturity of iPSC-CMs used. Consistent with this idea the same group also reported that the sequestration rate of the thapsigargin-sensitive caffeine-triggered store in hiPSC-CM was significantly slower than that of rabbit or mouse cardiomyocytes, [34]. Yet others have reported very small caffeine induced Ca^{2+} release stores even when compared to human embryonic stem cell derived myocytes [28]. Given the variation in technics of creating hiPSC-CM cell-lines, the level of their maturity, the degree of Ca^{2+} buffering and concentrations of Ca^{2+}-sensitive dyes used in intact or whole-cell clamped myocytes, it is likely that the quantitative variations in Ca^{2+} signaling parameters result from both developmental and electrophysiological approaches used.

2.3. Calcium Sparks and sporadic focal Ca^{2+} releases

Our experiments, using rapid (120–240 f/s) 2-D confocal imaging, show that the majority of sporadic and brief Ca^{2+}-sparks and focal releases in hiPSC-CMs had spatiotemporal properties analogous to those of adult rat cardiomyocytes, Ca^{2+} spark durations rarely exceeded 20 ms in control hiPSC-CM [33]. Others using line-scan imaging, have identified stochastic and repetitive Ca^{2+} sparks that re-occurred at the same site in hiPSC-CM. Ca^{2+} sparks appear to be predominately triggered by activation of L-type Ca^{2+} channels, and are enhanced in frequency on elevation of extracellular Ca^{2+}. Ca^{2+} diffusion from the center of Ca^{2+} sparks to periphery appears often to be asymmetric [29].

2.4. IP_3-gated Ca^{2+} pools

Although the primary Ca^{2+}-signaling pathway in adult mammalian cardiomyocytes occurs through activation of RYR2, in some myocytes and at different stages of their development there appears to be significant contribution from the IP_3-gated signaling pathway [46] [47]. IP_3R-gated Ca^{2+} release is expressed both in endoplasmic reticulum and the nuclear envelop [48]. The functional role of IP_3R in cardiomyocytes remains poorly understood. One study shows that IP_3R mRNA levels were about 50-fold lower than those of RYR2 mRNA in adult cardiac myocytes [14], nevertheless there is abundant expression of this protein in embryonic, neonatal, and adult atrial myocytes [18, 49–52]. In fact, it has been reported that the spontaneous electrical activity of embryonic pacemaker and stem cell-derived cardiomyocytes depends, in part, on IP_3-mediated Ca^{2+} release [53–55]. Since IP_3-gated Ca^{2+} signaling is reported to trigger both sub-sarcolemmal and perinuclear Ca^{2+} releases in developing cardiomyocytes [18], it is more likely that the sub-sarcolemmal Ca^{2+} release would activate the Na^+/Ca^{2+} exchanger to depolarize the surface membrane, while Ca^{2+} released at the nuclear envelop might have higher likelihood to be sequestered by SERCA2a [56]. Immuno-cyto-staining studies also suggest that IP_3R is mostly distributed around the

nucleus and that this pool may contribute to the modulation of Ca^{2+}-signaling in hiPSC-CM [57] [40]. Consistent with this idea IP_3R antagonist 2-APB or phospholipase C inhibitor U73122 have been reported to significantly slow the kinetics and decrease the amplitude of whole cell Ca^{2+} transients [40]. We have not been able to confirm this finding in our hiPSC-CM and neonatal cardiomyocytes, and in sharp contrast find that Ca^{2+} release from the SR and mitochondria might be the primary mechanism for the spontaneous pacing activity. In our study U73122 only slightly affected the beating frequency of hiPSC-CM, suggesting that IP_3R Ca^{2+} signaling played a minor role in generation of Ca^{2+} transients and regulation of pacing [33].

2.5. Mitochondrial Ca^{2+} signaling

There is little or no information as yet on mitochondrial Ca^{2+} signaling in hiPSC-CM. The contribution of mitochondrial Ca^{2+} signaling to spontaneous beating activity of hiPSC-CM has been examined recently using genetically encoded mitochondrial probes targeted to mitochondrial subunit VIII of cytochrome C. These studies showed both release and uptake of Ca^{2+} by different mitochondrial populations in hiPSC-CM; generally, though not always, perinuclear mitochondrial population released Ca^{2+} while the peripheral mitochondria took-up Ca^{2+} (Fig. 3, traces and panels 1,2,3). FCCP, a mitochondrial uncoupler, at 50nM suppressed the spontaneous beating of hiPSC-CM and prolonged the relaxation time of caffeine-induced Ca^{2+} transients, suggestive of a direct role for mitochondria in the cycling of cytosolic Ca^{2+} and regulation of pacing, (fig 3, trace 4 and panel 4). Our results therefore support a novel role for rapid release of Ca^{2+} from mitochondria in generation and regulation of spontaneous pacing [33].

3. Ca^{2+} signaling profiles of catecholaminergic polymorphic ventricular tachycardia (CPVT1) in hiPSC-CM model: “from mice to men”

Two genetic forms of CPVT have been thus far identified: 1) CPVT1, with point mutations in RyR2, accounting for at least 50% of all cases, resulting from abnormal intracellular Ca^{2+} handling caused by autosomal dominant mutations in the RYR2 gene [58] [59]; and 2) CPVT2, with an autosomal recessive mutations in cardiac calsequestrin, CASQ2, isoform also resulting in Ca^{2+} signaling defect [60].

CPVT1 causing mutations have been extensively studied in transgenic mice models or transformed cell lines. Evidence from a number of labs suggest that CPVT1 results from PKA-mediated phosphorylation of RyR2/FKBP complex resulting in SR diastolic Ca^{2+} leak that produces local depolarizations that trigger DADs via activation of NCX [61]. It has also been proposed that SR Ca^{2+} overload and altered Ca^{2+} sensitivity of SR luminal sites, not related to FKBP modulation, can cause diastolic Ca^{2+} release from RyR2, leading to local depolarizations and arrhythmogenesis [62] These opposing views are in part fueled by multiplicity of point mutations that cause CPVT1, and the variety of genetically engineered mice and animal cell-lines used. The iPSC technology has made it possible to create human cardiomyocyte cell-lines that express CPVT1 causing mutations. The general approach has been to obtain dermal fibroblasts from patients expressing CPVT1 arrhythmias, reprogramming and driving them into hiPSC-CM lineage [27]. To date, over 150 mutations

have been identified in RyR2 gene that causes CPVT1 [63] and 11 patient-specific CPVT1 hiPSC-CM cell lines have been generated and evaluated by several labs (Table 2). Since Ca^{2+} signaling pathway defects play critical roles in many cardiac pathologies, accurate knowledge as to the specific details of Ca^{2+} signaling defects in various CPVT1 models is required to provide insights into the mechanisms underlying the particular form of the disease and help design approaches for it in *vitro* pharmacotherapy, before applying the therapy to patients. Table 2 summarizes the Ca^{2+} signaling phenotypes of CPVT1 causing mutations as expressed in hiPSC-CM cell-lines, as depicted in the cartoon of RyR2 (Fig. 4). Only few studies appear to have attempted simultaneous measurements of membrane currents and Ca^{2+} signaling parameters beyond measurements of Ca^{2+} transients. The parameters required for full analysis of Ca^{2+} signaling must include: the density of I_{Ca} and I_{NCX}, the characteristics of I_{Ca}-gated Ca^{2+} release, the gain of CICR, Ca^{2+} storage capacity of the SR and its fractional release, the unitary properties of Ca^{2+} sparks and their frequency of occurrence, in addition to frequency of spontaneously triggered DADs or EADs, the generation of aberrant Ca^{2+} transients, and the alterations caused by application of isoproterenol. For instance, in hiPSC-CM from a patient expressing point mutation F2483I in RyR2, the caffeine-triggered Ca^{2+} transients and accompanying I_{NCX} were found to be significantly smaller than in control myocytes (Fig. 2D), consistent with a smaller SR Ca^{2+} store, possibly due to Leaky RyR2 [33]. Interestingly the smaller store of Ca^{2+} appeared to have been in part compensated for by a larger fractional release of Ca^{2+} triggered by I_{Ca}. Thus, the gain of CICR was higher in F2483I mutant expressing hiPSC-CM. As might be expected rapid electrical pacing, beta-adrenergic stimulation, or higher intracellular Na^{+} exacerbated Ca^{2+} handling abnormalities of F2483I mutant cells and increased the diastolic Ca^{2+} levels, thereby triggering spontaneously occurring Ca^{2+} transients [33]. Confocal Ca^{2+} imaging also showed that the F2483I mutants exhibit larger and more recurrent and spatially wandering sparks compared to sporadic and brief sparks of control iPSC-CM, especially under Ca^{2+}-overload conditions. Often, the Ca^{2+}-induced Ca^{2+}-release events continued after repolarization in CPVT1-CMs suggesting slower decay of Ca^{2+} transients, Fig. 2B, consistent with the continued release of Ca^{2+}. A large fraction (59%) of CPVT-CMs also showed slower frequency of spontaneous beating and 34% developed arrhythmia and DAD after Isoproterenol stimulation [27].

In the N-terminal segment, two CPVT1 causing point mutations have been reported in human cell lines, fig. 4. Novak *et al.* [44] reported that R420Q mutation exhibited immature ultra-structural features including poorly developed SR, poorly organized myofibrillar sarcomeric pattern, and higher mitochondrial expression. They also report 60% slower spontaneous beating rates as compared to control hiPSC-CM. Caffeine induced Ca^{2+} transients were smaller and shorter, consistent with smaller SR Ca^{2+} stores but with faster reuptake of intracellular Ca^{2+}. Even though isoproterenol increased the amplitude and maximal rate of rise and fall of Ca^{2+} transients in control cells, three types of responses were found in R420Q mutant cells: 1) 59% of CPVT1-CM were irresponsive to isoproterenol; 2) 16% CPVT1-CM showed arrhythmogenic responses; and 3) in 25% of CPVT-CMs isoproterenol decreased Ca^{2+} transients and elevated diastolic Ca^{2+} levels, Table 2. In another N-terminal CPTV1-causing mutation, S406L [64], although cardiomyocytes appeared to have similar diastolic and systolic Ca^{2+} levels and comparable SR Ca^{2+} content

under basal condition as control myocytes, in the presence of isoproterenol the diastolic Ca^{2+}, the frequency of Ca^{2+} sparks, and the susceptibility to DADs and arrhythmia increased, even though systolic Ca^{2+} and SR Ca^{2+} content remained unaltered [64]. Interestingly, high frequency electrical pacing also increased the percentage of cells with abnormal Ca^{2+} handling. These authors report that dantrolene restored Ca^{2+} sparks frequency in CPVT mutant cells both under basal and adrenergic stimulated conditions, and abolished DAD and triggered arrhythmias [64]. Comparison of Ca^{2+} content of these two point mutations in the N-terminal domain suggests a decrease in R420Q, consistent with RyR2 leak hypothesis, and no change in S406L, possibly supporting increased luminal Ca^{2+} sensitivity of SR, Table 2.

Four hiPSC-CM cell lines have been thus far created carrying CPVT point mutations flanking FKBP domain in the cytosolic region of RyR2, Fig 4. In E2311D cell-line Ca^{2+} transients appeared to initiate from multiple loci and isoproterenol increased the number of sites triggering the spontaneous Ca^{2+} transients. Under basal conditions, 43% CPVT-CMs developed DAD during the diastolic depolarization phase. 12% of the cells also developed DADs even when paced at low frequencies of 0.5Hz, and the DAD frequencies increased significantly after β-adrenergic stimulation. Interestingly, CaMKII inhibitor KN-93 stabilized the Ca^{2+} activity and suppressed isoproterenol-induced DAD in this model [65]. The P2328S CPVT1 causing mutation cell-line [43] also displays aberrant Ca^{2+} cycling, decreased Ca^{2+} content, and a fractional Ca^{2+} release that was significantly higher both in control and isoproterenol-treated myocytes. In this cell-line both EAD and DAD were consistently recorded and their frequency was increased on adrenergic stimulation.

Penttinen *et al.* [66] also developed 6 CPVT cell lines with different mutations located in different parts of the RyR2 protein from N-terminal, to central, to cytoplasmic, to C-terminal domains (Fig. 4) that included: Exon-3 deletion and point mutations T2538R, P2328S, Q420I, L4115F and V4653F. All cell lines showed abnormalities in Ca^{2+} transients and spontaneous beating frequency both under basal conditions and adrenergic stimulation, but diastolic Ca^{2+} was only increased in P2328S CPVT-CMs. Unfortunately there is no data on Ca^{2+} content of SR or its fractional release in five of six mutant cell-lines, providing no mechanistic support for either of the two CPVT1 hypothesis, Table 2. Nevertheless, pharmacotherapy with dantrolene was effective in suppressing Ca^{2+} cycling abnormalities only in cells having the RyR2 mutations in the N-terminal and central regions, but not in transmembrane domains, consistent with the antiarrhythmic effect of this drug in patients with RyR2 mutations in the N-terminal or central regions of RyR2 protein [66].

Itzhaki *et al.* also generated a hiPSC-CM heterozygous cell line carrying point mutation at M4109R from a CPVT patient [67]. These myocytes also show abnormal Ca^{2+} transients and appeared to develop store-overload induced Ca^{2+} release (SOICR) at much lower Ca^{2+} concentrations than the healthy control cells. In this cell-line, DADs developed in most cells even when paced at 0.5 to 1 Hz. Adrenergic stimulation significantly increased the frequency and magnitude of DADs and flecainide and thapsigargin significantly suppressed the incidence of DAD [67].

4. Conclusion and Perspectives

Although there are many descriptive electrophysiological studies and measurements of Ca^{2+} transients in hiPSC-CM cell-lines, there are only few reports that have quantified the Ca^{2+} signaling parameters ad their regulation in whole-cell patch clamped myocytes. In few studies where hiPSC-CM cell-lines, created either from normal subjects or CPVT patients, were studied in greater detail using confocal and TIRF imaging in voltage-clamped cells, the data suggest that all the parameters of mammalian cardiac signaling (i.e., phenotypic bell-shaped voltage-dependence of I_{Ca}-gated Ca^{2+} release and its voltage-dependent gain, spontaneously triggered Ca^{2+} sparks, functional levels of NCX gene, β-adrenergic regulation of I_{Ca}, and SERCA2a/PLB complex) are expressed in human fibroblast-derived cardiomyocytes. It is, therefore, fair to conclude that hiPSC-CM can serve as a reliable model for human cardiomyocytes, but care must be exercised to characterize fully the various electrophysiological and Ca^{2+} signaling parameters of each new hiPSC-CM cell line before pharmacological and pathophysiological studies are undertaken.

Clearly, there appears to be significant variations on the degree of maturity of cell-lines generated by different labs that may underlie some of the observed differences in the reported Ca^{2+} content of SR, their beta-adrenergic modulation, the systolic and diastolic Ca^{2+} levels, and their spontaneous beating rates. It is not as yet clear whether such variations arise solely from developmental stages of created hiPSC-CM or are related to various cellular biological approaches and *in vitro* experimental conditions modifying the physiological signaling pathways.

This review also compares the Ca^{2+} signaling parameters of hiPSC-CMs of control subjects to those derived from patients expressing CPVT1, Table 2. The mutations expressed in various hiPSC-CM cell lines are located in the well-known CPVT hotspots of the RyR2 gene. All mutations appear to cause aberrant Ca^{2+} transients and arrhythmias. Ca^{2+} transient abnormalities were somewhat more common in mutations located in transmembrane domain than in central and N-terminal regions, and were preferentially suppressed by dantrolene [64, 66]. Exon 3 deleted hiPSC-CMs had both lower beating frequencies and diastolic Ca^{2+} levels when compared to other mutations, possibly resulting from altered secondary structures critical to folding of the N-terminal domain and its conformational changes [68]. There also appears to be significant variability in myocyte responsiveness to β-adrenergic stimulation in various cell lines expressing CPVT-inducing mutations. For instance, β-adrenergic agonists failed to increase the diastolic Ca^{2+} in Exon-3 deleted cells, as well as in cell lines expressing T2538R, L4115F, Q4201R and V4653F, as compared to S406L, R420Q, P2328S, F2483I, which in sharp contrast have increased diastolic Ca^{2+} levels on exposure to Isoproterenol. Given the inter cellular variability of hiPSC-CMs' responsiveness to beta agonists it becomes difficult to develop a comprehensive hypothesis as to the mechanisms responsible for development of CPVT on the global whole-heart levels, but this variability may underlie the intriguing finding that the occurrence of CPVT in patients are generally episodic and often unpredictable on exertion or adrenergic stimulation.

CPVT cell lines expressing mutations of different RyR2 loci also show some diversity in the SR Ca^{2+} content. In 4 of the 11 mutant CPVT1 human cell lines, thus far reported, the Ca^{2+}

content of SR was smaller, consistent with “leaky” RyR2s, Table 2, but at least in F2483I cell line (located in the vicinity of FKBP binding site) the decreased Ca^{2+} content is accompanied by higher I_{Ca}-gated fractional Ca^{2+} release. In this respect, it appears to be a common finding that the rate of spontaneous beating is lower in CPVT1 mutant cell lines as compared to control lines, possibly because of the lower SR Ca^{2+} content. This finding is consistent with the hypothesis that Ca^{2+} signaling mechanism, rather than the activation of I_f regulates spontaneous pacing activity in hiPSC-CMs [45].

Given the inter-cellular variability in adrenergic responsiveness and diversity in Ca^{2+} content of SR in different CPVT cell lines, perhaps more sophisticated approaches of simultaneous mapping of both electrical and calcium signals in monolayers of CPVT-expressing cell lines should be combined with single cell studies in identifying and quantifying the possible defects of Ca^{2+} signaling pathways. These are difficult issues to sort out, but they must be done if iPSC-derived human cardiac cell lines are to be used reliably, especially in pharmacotherapeutic studies. Clearly there are differences in the cellular phenotype, the functional pathology, and the pharmacology of different CPVT1 cell-lines. This, we believe, bodes well for the future of this field especially as more detailed characterizations of these cell-lines and their sensitivity to various pharmacotherapies are undertaken.

Acknowledgments

We thank Drs. Naohiro Yamaguchi and Lars Cleemann for helpful suggestions. Supported by NIH R01 HL16152.

References

1. Morad M, Trautwein W. The effect of the duration of the action potential on contraction in the mammalian heart muscle. Pflugers Archiv fur die gesamte Physiologie des Menschen und der Tiere. 1968; 299:66–82.
2. Morad M, Orkand RK. Excitation-concentration coupling in frog ventricle: evidence from voltage clamp studies. The Journal of physiology. 1971; 219:167–189. [PubMed: 5316660]
3. Grynkiewicz G, Poenie M, Tsien RY. A new generation of Ca2+ indicators with greatly improved fluorescence properties. The Journal of biological chemistry. 1985; 260:3440–3450. [PubMed: 3838314]
4. Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflugers Archiv : European journal of physiology. 1981; 391:85–100. [PubMed: 6270629]
5. Nabauer M, Callewaert G, Cleemann L, Morad M. Regulation of calcium release is gated by calcium current, not gating charge, in cardiac myocytes. Science. 1989; 244:800–803. [PubMed: 2543067]
6. Fabiato A. Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. The American journal of physiology. 1983; 245:C1–14. [PubMed: 6346892]
7. Cheng H, Lederer WJ, Cannell MB. Calcium sparks: elementary events underlying excitation-contraction coupling in heart muscle. Science. 1993; 262:740–744. [PubMed: 8235594]
8. Cleemann L, Wang W, Morad M. Two-dimensional confocal images of organization, density, and gating of focal Ca2+ release sites in rat cardiac myocytes. Proceedings of the National Academy of Sciences of the United States of America. 1998; 95:10984–10989. [PubMed: 9724816]
9. Barcenas-Ruiz L, Wier WG. Voltage dependence of intracellular [Ca2+]i transients in guinea pig ventricular myocytes. Circulation research. 1987; 61:148–154. [PubMed: 2440616]
10. Adachi-Akahane S, Cleemann L, Morad M. Cross-signaling between L-type Ca2+ channels and ryanodine receptors in rat ventricular myocytes. The Journal of general physiology. 1996; 108:435–454. [PubMed: 8923268]

11. Woo SH, Cleemann L, Morad M. Spatiotemporal characteristics of junctional and nonjunctional focal Ca2+ release in rat atrial myocytes. Circulation research. 2003; 92:e1–11. [PubMed: 12522129]
12. Morad M, Cleemann L. Tunicate heart as a possible model for the vertebrate heart. Federation proceedings. 1980; 39:3188–3194. [PubMed: 7192220]
13. Bers DM. Calcium cycling and signaling in cardiac myocytes. Annual review of physiology. 2008; 70:23–49.
14. Moschella MC, Marks AR. Inositol 1,4,5-trisphosphate receptor expression in cardiac myocytes. The Journal of cell biology. 1993; 120:1137–1146. [PubMed: 8382205]
15. Puceat M, Jaconi M. Ca2+ signalling in cardiogenesis. Cell calcium. 2005; 38:383–389. [PubMed: 16099501]
16. Webb SE, Miller AL. Calcium signalling during embryonic development. Nature reviews Molecular cell biology. 2003; 4:539–551. [PubMed: 12838337]
17. Kume S, Yamamoto A, Inoue T, Muto A, Okano H, Mikoshiba K. Developmental expression of the inositol 1,4,5-trisphosphate receptor and structural changes in the endoplasmic reticulum during oogenesis and meiotic maturation of Xenopus laevis. Developmental biology. 1997; 182:228–239. [PubMed: 9070324]
18. Janowski E, Berrios M, Cleemann L, Morad M. Developmental aspects of cardiac Ca(2+) signaling: interplay between RyR- and IP(3)R-gated Ca(2+) stores. American journal of physiology Heart and circulatory physiology. 2010; 298:H1939–1950. [PubMed: 20304819]
19. Nakayama H, Bodi I, Maillet M, DeSantiago J, Domeier TL, Mikoshiba K, Lorenz JN, Blatter LA, Bers DM, Molkentin JD. The IP3 receptor regulates cardiac hypertrophy in response to select stimuli. Circulation research. 2010; 107:659–666. [PubMed: 20616315]
20. Go LO, Moschella MC, Watras J, Handa KK, Fyfe BS, Marks AR. Differential regulation of two types of intracellular calcium release channels during end-stage heart failure. The Journal of clinical investigation. 1995; 95:888–894. [PubMed: 7860772]
21. Barac YD, Zeevi-Levin N, Yaniv G, Reiter I, Milman F, Shilkrut M, Coleman R, Abassi Z, Binah O. The 1,4,5-inositol trisphosphate pathway is a key component in Fas-mediated hypertrophy in neonatal rat ventricular myocytes. Cardiovascular research. 2005; 68:75–86. [PubMed: 16005448]
22. Carafoli E, Lehninger AL. Binding of adenine nucleotides by mitochondria during active uptake of CA++ Biochemical and biophysical research communications. 1964; 16:66–70. [PubMed: 5871793]
23. Dedkova EN, Blatter LA. Mitochondrial Ca2+ and the heart. Cell calcium. 2008; 44:77–91. [PubMed: 18178248]
24. Barth E, Stammler G, Speiser B, Schaper J. Ultrastructural quantitation of mitochondria and myofilaments in cardiac muscle from 10 different animal species including man. Journal of molecular and cellular cardiology. 1992; 24:669–681. [PubMed: 1404407]
25. Belmonte S, Morad M. 'Pressure-flow'-triggered intracellular Ca2+ transients in rat cardiac myocytes: possible mechanisms and role of mitochondria. The Journal of physiology. 2008; 586:1379–1397. [PubMed: 18187469]
26. Haviland S, Cleemann L, Kettlewell S, Smith GL, Morad M. Diversity of mitochondrial Ca(2)(+) signaling in rat neonatal cardiomyocytes: evidence from a genetically directed Ca(2)(+) probe, mitycam-E31Q. Cell calcium. 2014; 56:133–146. [PubMed: 24994483]
27. Fatima A, Xu G, Shao K, Papadopoulos S, Lehmann M, Arnaiz-Cot JJ, Rosa AO, Nguemo F, Matzkies M, Dittmann S, Stone SL, Linke M, Zechner U, Beyer V, Hennies HC, Rosenkranz S, Klauke B, Parwani AS, Haverkamp W, Pfitzer G, Farr M, Cleemann L, Morad M, Milting H, Hescheler J, Saric T. In vitro modeling of ryanodine receptor 2 dysfunction using human induced pluripotent stem cells. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology. 2011; 28:579–592.
28. Lee YK, Ng KM, Lai WH, Chan YC, Lau YM, Lian Q, Tse HF, Siu CW. Calcium homeostasis in human induced pluripotent stem cell-derived cardiomyocytes. Stem cell reviews. 2011; 7:976–986. [PubMed: 21614516]

29. Zhang GQ, Wei H, Lu J, Wong P, Shim W. Identification and characterization of calcium sparks in cardiomyocytes derived from human induced pluripotent stem cells. PloS one. 2013; 8:e55266. [PubMed: 23408964]
30. Lundy SD, Zhu WZ, Regnier M, Laflamme MA. Structural and functional maturation of cardiomyocytes derived from human pluripotent stem cells. Stem cells and development. 2013; 22:1991–2002. [PubMed: 23461462]
31. Gherghiceanu M, Barad L, Novak A, Reiter I, Itskovitz-Eldor J, Binah O, Popescu LM. Cardiomyocytes derived from human embryonic and induced pluripotent stem cells: comparative ultrastructure. Journal of cellular and molecular medicine. 2011; 15:2539–2551. [PubMed: 21883888]
32. Lieu DK, Liu J, Siu CW, McNerney GP, Tse HF, Abu-Khalil A, Huser T, Li RA. Absence of transverse tubules contributes to non-uniform Ca(2+) wavefronts in mouse and human embryonic stem cell-derived cardiomyocytes. Stem cells and development. 2009; 18:1493–1500. [PubMed: 19290776]
33. Zhang XH, Haviland S, Wei H, Saric T, Fatima A, Hescheler J, Cleemann L, Morad M. Ca2+ signaling in human induced pluripotent stem cell-derived cardiomyocytes (iPS-CM) from normal and catecholaminergic polymorphic ventricular tachycardia (CPVT)-afflicted subjects. Cell calcium. 2013; 54:57–70. [PubMed: 23684427]
34. Hwang HS, Kryshtal DO, Feaster TK, Sanchez-Freire V, Zhang J, Kamp TJ, Hong CC, Wu JC, Knollmann BC. Comparable calcium handling of human iPSC-derived cardiomyocytes generated by multiple laboratories. Journal of molecular and cellular cardiology. 2015; 85:79–88. [PubMed: 25982839]
35. Ma J, Guo L, Fiene SJ, Anson BD, Thomson JA, Kamp TJ, Kolaja KL, Swanson BJ, January CT. High purity human-induced pluripotent stem cell-derived cardiomyocytes: electrophysiological properties of action potentials and ionic currents. American journal of physiology Heart and circulatory physiology. 2011; 301:H2006–2017. [PubMed: 21890694]
36. Morad M, Goldman Y. Excitation-contraction coupling in heart muscle: Membrane control of development of tension Progress in biophysics and molecular biology. 1973; 27:257–313.
37. Morad M, Cleemann L. Role of Ca2+ channel in development of tension in heart muscle. Journal of molecular and cellular cardiology. 1987; 19:527–553. [PubMed: 2442398]
38. Cleemann L, Morad M. Role of Ca2+ channel in cardiac excitation-contraction coupling in the rat: evidence from Ca2+ transients and contraction. The Journal of physiology. 1991; 432:283–312. [PubMed: 1653321]
39. Chen F, Mottino G, Klitzner TS, Philipson KD, Frank JS. Distribution of the Na+/Ca2+ exchange protein in developing rabbit myocytes. The American journal of physiology. 1995; 268:C1126–1132. [PubMed: 7762604]
40. Itzhaki I, Rapoport S, Huber I, Mizrahi I, Zwi-Dantsis L, Arbel G, Schiller J, Gepstein L. Calcium handling in human induced pluripotent stem cell derived cardiomyocytes. PloS one. 2011; 6:e18037. [PubMed: 21483779]
41. Germanguz I, Sedan O, Zeevi-Levin N, Shtrichman R, Barak E, Ziskind A, Eliyahu S, Meiry G, Amit M, Itskovitz-Eldor J, Binah O. Molecular characterization and functional properties of cardiomyocytes derived from human inducible pluripotent stem cells. Journal of cellular and molecular medicine. 2011; 15:38–51. [PubMed: 20041972]
42. Shmigol A, Verkhratsky A, Isenberg G. Calcium-induced calcium release in rat sensory neurons. The Journal of physiology. 1995; 489(Pt 3):627–636. [PubMed: 8788929]
43. Kujala K, Paavola J, Lahti A, Larsson K, Pekkanen-Mattila M, Viitasalo M, Lahtinen AM, Toivonen L, Kontula K, Swan H, Laine M, Silvennoinen O, Aalto-Setala K. Cell model of catecholaminergic polymorphic ventricular tachycardia reveals early and delayed afterdepolarizations. PloS one. 2012; 7:e44660. [PubMed: 22962621]
44. Novak A, Barad L, Lorber A, Gherghiceanu M, Reiter I, Eisen B, Eldor L, Itskovitz-Eldor J, Eldar M, Arad M, Binah O. Functional abnormalities in iPSC-derived cardiomyocytes generated from CPVT1 and CPVT2 patients carrying ryanodine or calsequestrin mutations. Journal of cellular and molecular medicine. 2015; 19:2006–2018. [PubMed: 26153920]

45. Zhang XH, Wei H, Saric T, Hescheler J, Cleemann L, Morad M. Regionally diverse mitochondrial calcium signaling regulates spontaneous pacing in developing cardiomyocytes. Cell calcium. 2015; 57:321–336. [PubMed: 25746147]

46. Rosemblit N, Moschella MC, Ondriasa E, Gutstein DE, Ondrias K, Marks AR. Intracellular calcium release channel expression during embryogenesis. Developmental biology. 1999; 206:163–177. [PubMed: 9986730]

47. Marks AR. Intracellular calcium-release channels: regulators of cell life and death. The American journal of physiology. 1997; 272:H597–605. [PubMed: 9124414]

48. Rizzuto R, Pozzan T. Microdomains of intracellular Ca2+: molecular determinants and functional consequences. Physiological reviews. 2006; 86:369–408. [PubMed: 16371601]

49. Luo D, Yang D, Lan X, Li K, Li X, Chen J, Zhang Y, Xiao RP, Han Q, Cheng H. Nuclear Ca2+ sparks and waves mediated by inositol 1,4,5-trisphosphate receptors in neonatal rat cardiomyocytes. Cell calcium. 2008; 43:165–174. [PubMed: 17583790]

50. Sedan O, Dolnikov K, Zeevi-Levin N, Leibovich N, Amit M, Itskovitz-Eldor J, Binah O. 1,4,5-Inositol trisphosphate-operated intracellular Ca(2+) stores and angiotensin-II/endothelin-1 signaling pathway are functional in human embryonic stem cell-derived cardiomyocytes. Stem cells. 2008; 26:3130–3138. [PubMed: 18818435]

51. Satin J, Itzhaki I, Rapoport S, Schroder EA, Izu L, Arbel G, Beyar R, Balke CW, Schiller J, Gepstein L. Calcium handling in human embryonic stem cell-derived cardiomyocytes. Stem cells. 2008; 26:1961–1972. [PubMed: 18483424]

52. Kim JC, Son MJ, Subedi KP, Li Y, Ahn JR, Woo SH. Atrial local Ca2+ signaling and inositol 1,4,5-trisphosphate receptors. Progress in biophysics and molecular biology. 2010; 103:59–70. [PubMed: 20193706]

53. Sasse P, Zhang J, Cleemann L, Morad M, Hescheler J, Fleischmann BK. Intracellular Ca2+ oscillations, a potential pacemaking mechanism in early embryonic heart cells. The Journal of general physiology. 2007; 130:133–144. [PubMed: 17664344]

54. Kapur N, Banach K. Inositol-1,4,5-trisphosphate-mediated spontaneous activity in mouse embryonic stem cell-derived cardiomyocytes. The Journal of physiology. 2007; 581:1113–1127. [PubMed: 17379641]

55. Mery A, Aimond F, Menard C, Mikoshiba K, Michalak M, Puceat M. Initiation of embryonic cardiac pacemaker activity by inositol 1,4,5-trisphosphate-dependent calcium signaling. Molecular biology of the cell. 2005; 16:2414–2423. [PubMed: 15758029]

56. Kapoor N, Maxwell JT, Mignery GA, Will D, Blatter LA, Banach K. Spatially defined InsP3-mediated signaling in embryonic stem cell-derived cardiomyocytes. PloS one. 2014; 9:e83715. [PubMed: 24409283]

57. Garcia KD, Shah T, Garcia J. Immunolocalization of type 2 inositol 1,4,5-trisphosphate receptors in cardiac myocytes from newborn mice. American journal of physiology Cell physiology. 2004; 287:C1048–1057. [PubMed: 15201137]

58. Priori SG, Napolitano C, Tiso N, Memmi M, Vignati G, Bloise R, Sorrentino V, Danieli GA. Mutations in the cardiac ryanodine receptor gene (hRyR2) underlie catecholaminergic polymorphic ventricular tachycardia. Circulation. 2001; 103:196–200. [PubMed: 11208676]

59. Novak A, Lorber A, Itskovitz-Eldor J, Binah O. Modeling Catecholaminergic Polymorphic Ventricular Tachycardia using Induced Pluripotent Stem Cell-derived Cardiomyocytes. Rambam Maimonides medical journal. 2012; 3:e0015. [PubMed: 23908839]

60. Postma AV, Denjoy I, Hoorntje TM, Lupoglazoff JM, Da Costa A, Sebillon P, Mannens MM, Wilde AA, Guicheney P. Absence of calsequestrin 2 causes severe forms of catecholaminergic polymorphic ventricular tachycardia. Circulation research. 2002; 91:e21–26. [PubMed: 12386154]

61. Wehrens XH, Lehnart SE, Huang F, Vest JA, Reiken SR, Mohler PJ, Sun J, Guatimosim S, Song LS, Rosemblit N, D'Armiento JM, Napolitano C, Memmi M, Priori SG, Lederer WJ, Marks AR. FKBP12.6 deficiency and defective calcium release channel (ryanodine receptor) function linked to exercise-induced sudden cardiac death. Cell. 2003; 113:829–840. [PubMed: 12837242]

62. Jiang D, Wang R, Xiao B, Kong H, Hunt DJ, Choi P, Zhang L, Chen SR. Enhanced store overload-induced Ca2+ release and channel sensitivity to luminal Ca2+ activation are common defects of

RyR2 mutations linked to ventricular tachycardia and sudden death. Circulation research. 2005; 97:1173–1181. [PubMed: 16239587]

63. Lehnart SE, Mongillo M, Bellinger A, Lindegger N, Chen BX, Hsueh W, Reiken S, Wronska A, Drew LJ, Ward CW, Lederer WJ, Kass RS, Morley G, Marks AR. Leaky Ca2+ release channel/ryanodine receptor 2 causes seizures and sudden cardiac death in mice. The Journal of clinical investigation. 2008; 118:2230–2245. [PubMed: 18483626]
64. Jung CB, Moretti A, Mederos y Schnitzler M, Iop L, Storch U, Bellin M, Dorn T, Ruppenthal S, Pfeiffer S, Goedel A, Dirschinger RJ, Seyfarth M, Lam JT, Sinnecker D, Gudermann T, Lipp P, Laugwitz KL. Dantrolene rescues arrhythmogenic RYR2 defect in a patient-specific stem cell model of catecholaminergic polymorphic ventricular tachycardia. EMBO molecular medicine. 2012; 4:180–191. [PubMed: 22174035]
65. Di Pasquale E, Lodola F, Miragoli M, Denegri M, Avelino-Cruz JE, Buonocore M, Nakahama H, Portararo P, Bloise R, Napolitano C, Condorelli G, Priori SG. CaMKII inhibition rectifies arrhythmic phenotype in a patient-specific model of catecholaminergic polymorphic ventricular tachycardia. Cell death & disease. 2013; 4:e843. [PubMed: 24113177]
66. Penttinen K, Swan H, Vanninen S, Paavola J, Lahtinen AM, Kontula K, Aalto-Setala K. Antiarrhythmic Effects of Dantrolene in Patients with Catecholaminergic Polymorphic Ventricular Tachycardia and Replication of the Responses Using iPSC Models. PloS one. 2015; 10:e0125366. [PubMed: 25955245]
67. Itzhaki I, Maizels L, Huber I, Gepstein A, Arbel G, Caspi O, Miller L, Belhassen B, Nof E, Glikson M, Gepstein L. Modeling of catecholaminergic polymorphic ventricular tachycardia with patient-specific human-induced pluripotent stem cells. Journal of the American College of Cardiology. 2012; 60:990–1000. [PubMed: 22749309]
68. Lobo PA, Kimlicka L, Tung CC, Van Petegem F. The deletion of exon 3 in the cardiac ryanodine receptor is rescued by beta strand switching. Structure. 2011; 19:790–798. [PubMed: 21645850]
69. Gupta MK, Illich DJ, Gaarz A, Matzkies M, Nguemo F, Pfannkuche K, Liang H, Classen S, Reppel M, Schultze JL, Hescheler J, Saric T. Global transcriptional profiles of beating clusters derived from human induced pluripotent stem cells and embryonic stem cells are highly similar. BMC developmental biology. 2010; 10:98. [PubMed: 20843318]

Highlights

1. Specific carboxyl-tail fragments of L-type Ca^{2+} channel maybe responsible for the voltage-dependence of cardiac CICR.
2. Ca^{2+} signaling in human fibroblast-derived cardiomyocytes shows similar voltage-dependence of CICR and Ca^{2+} spark properties as those of adult mammalian cardiomyocytes.
3. CPVT mutants myocytes of N-terminal and cytoplasmic-domains show smaller Ca^{2+} stores but longer, wandering, and recurrent Ca^{2+} sparks.
4. Myocytes expressing different point mutations express varied Ca^{2+} signaling phenotypes and pharmacological sensitivity.

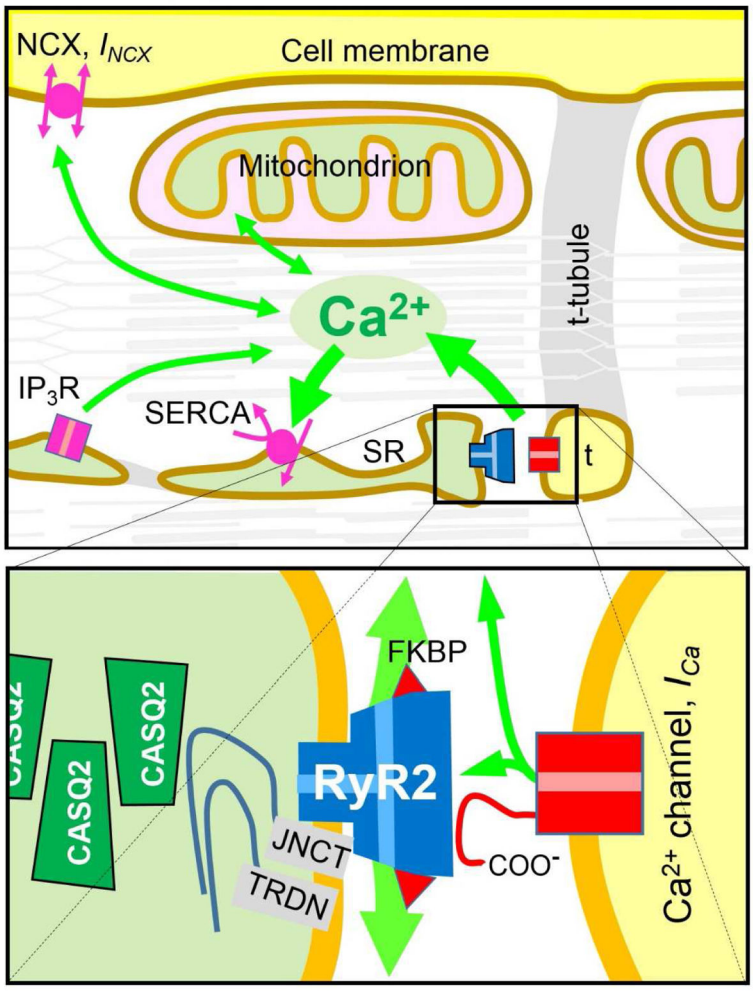


Figure 1.

Schematic of cardiac Ca^{2+} signaling. Top panel show Ca fluxes (green arrows) generated by dyadic junctions (box), Na^+/Ca^{2+} exchangers (NCX, I_{NCX}), sarco- and endoplasmic reticular Ca^{2+} ATPase (SERCA), inositol triphosphate receptor (IP_3R), and mitochondria in relation to the cell membrane, the transverse tubular system (t) and the sarcoplasmic reticulum (SR). All are shown superimposed on the contractile filaments to signify the regulation of cellular contraction and relaxation by cytosolic Ca^{2+}. The lower diagram shows key proteins within dyadic junctions where clusters of Ca^{2+} release channels (ryanodine receptors, RyR2) in the SR membrane are functionally and structurally associated with Ca^{2+} channels (DHP receptors, L-type Ca^{2+} current, I_{Ca}) in t-tubules, calsequestrin (CASQ2) in SR and, triadin (TRDN), junction (JNCT), and FKBP (calstabin) in association with RyR2.

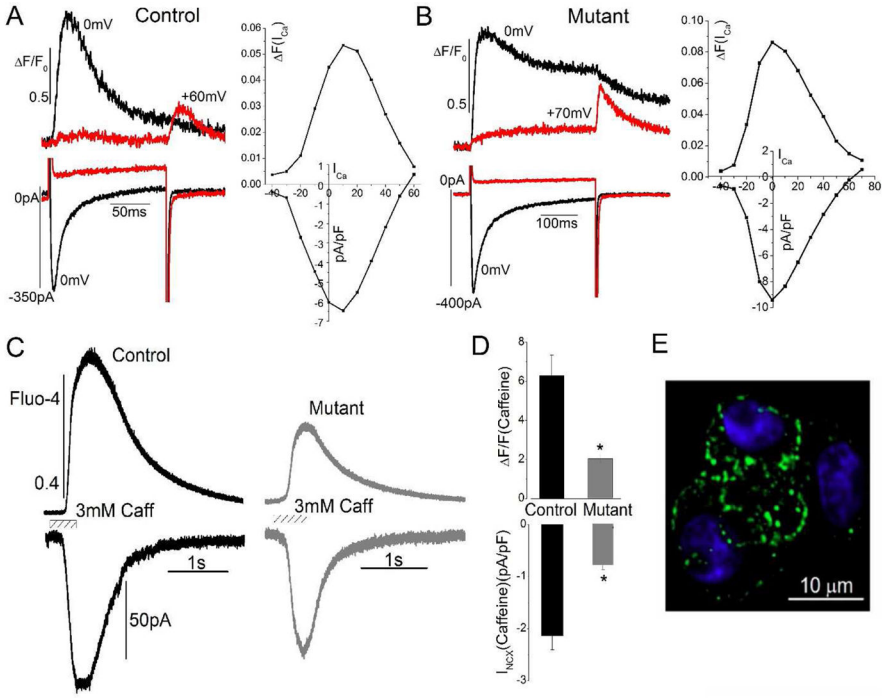


Figure 2.

Comparison of I_{Ca}- and caffeine-induced Ca^{2+} signals in hiPSC-CM from a healthy control subject and a CPVT1-afflicted patient with point mutation F2483I in RyR2. Currents were recorded with a 150ms or 250 ms step depolarizations from holding potential of −40 mV in 10 mV steps to +60 mV. (A) and (B) Left part of panels shows representative I_{Ca} and fluorescence traces where depolarization to 0mV rapidly activates Ca^{2+} release (black traces) while clamp pulses to +60 or +70 mV only activate rises in Ca^{2+} on repolarization (red traces). The right panel shows the I–V curves for I_{Ca} and the corresponding Ca^{2+} Fluo-4 signal from control and CPVT hiPSC-CM. (C) Representative caffeine-induced NCX currents and corresponding fluorescence Ca^{2+} signal from control and mutant hiPSC-CM. (D) Average values of caffeine-activated Ca^{2+} signals (top) and I_{NCX} currents (bottom) in each group. (E) Confocal image of immunofluorescence labeled RyR2 (green) in a small cluster of control hiPS-CM with DAPI-labeled nuclei (blue). Modified from [33]

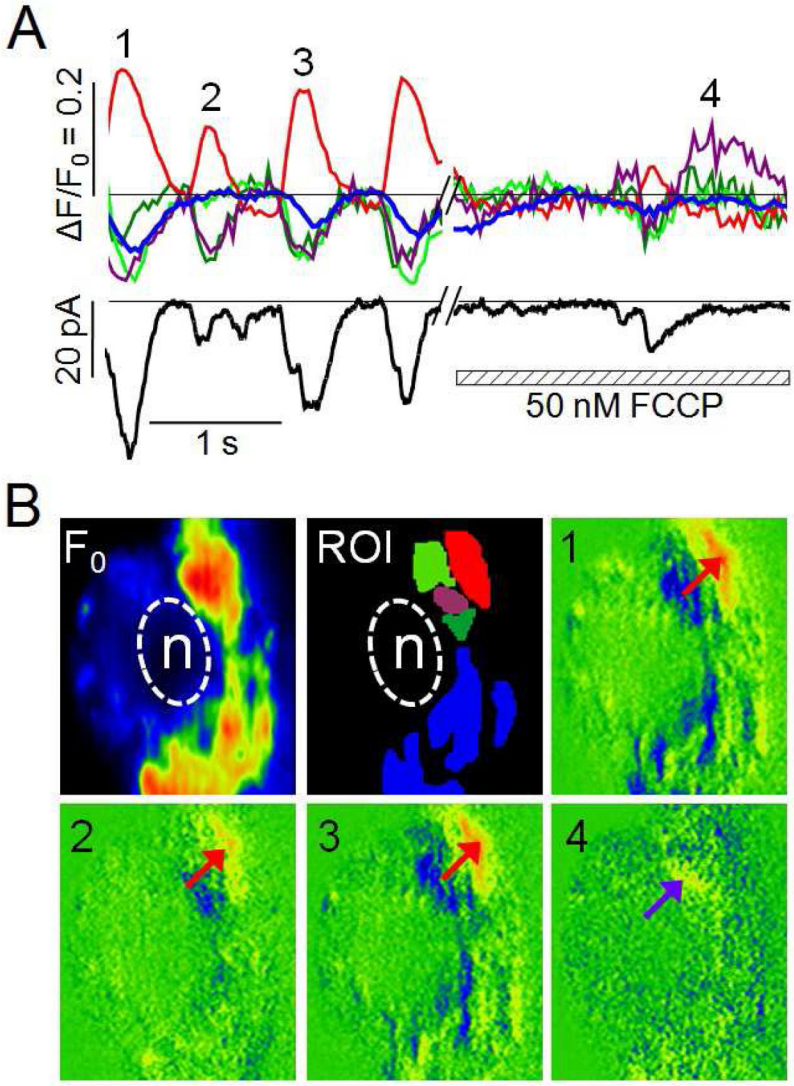


Figure 3.

Suppression of oscillatory mitochondrial Ca^{2+} signals in a spontaneously pacing control hiPSC-CM infected with the genetically engineered targeted, Ca^{2+} probe, myticam-E31Q [26]. (A) Tracings showing changes in regional mitochondrial Ca^{2+} signals (top) and I_{NCX} (bottom) before and 30 s after exposure to 50 nM FCCP. Increasing mitochondrial Ca^{2+} corresponds to downward deflection. (B) Images show baseline fluorescence (F_0) and color-coded regions of interest (ROI, with "n" indicating nuclei) and 4 differential fluorescence images (measured at the times indicated along the traces) showing repeatable mitochondrial Ca^{2+} signals before FCCP application (1, 2, 3) and smaller, more localized responses after (4). Modified from [45]

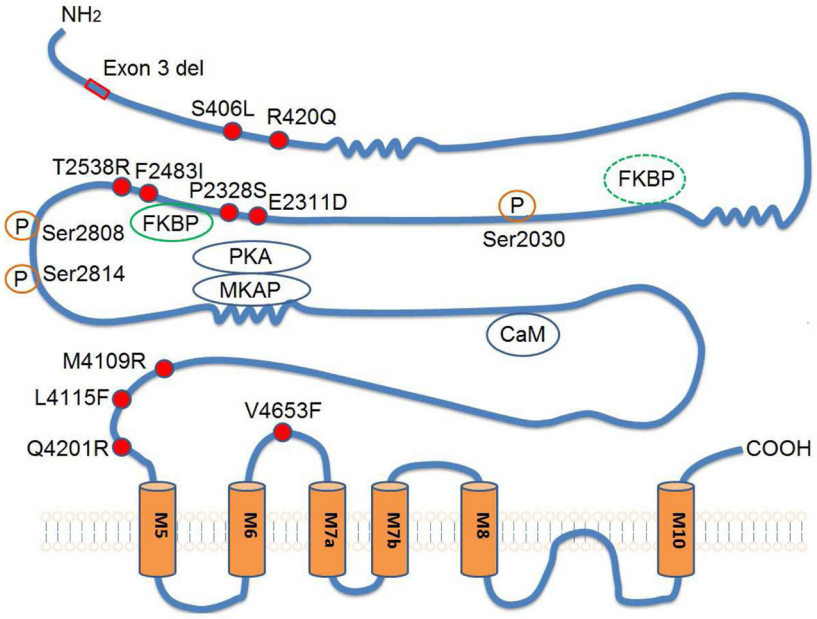


Figure 4.

Diagram of RyR2 receptor showing key functional domains in relation to 11 CPVT1-causing mutations that are located from N-terminal to central domain to C-terminal (red circles), which have been studied in hiPSC-CM. Functional domains: Trans-membrane α-helices (M#), phosphorylation sites (P), and binding sites for FKBP, calmodulin (CaM) and protein kinase A (PKA/MKAP) are also indicated.

Table 1

Calcium signaling characteristic of various hiPSC-CM cell lines.

hiPSC-CM Cell-lines	mRNA	Immunostaining	Action Potential	I_{Ca}	SR Ca^{2+} content	I_{NCX}	SERCA	Spontaneous Ca^{2+} transients	Ca^{2+} sparks	IP_3R
[69]	NKX2.5 MLC2v ACTN2 MYH6 GATA4 a	No data	No data	No data	++	No data	No data	++	No data	No data
[40]	RyR2, IP_3R2, SERCA2a Cav1.2 PLB; CSQ	α-actinin & RyR2	No data	No data	++++	No data	Thapsigargin suppress spontaneous and caffeine induced Ca^{2+} transients	suppressed by nifedipine or ryanodine	No data	2-APB or U73122 suppress Ca^{2+} transients
[28]	NCX1, RyR2, SERCA2a, MHC	α-actinin & TPM	45% N-CM, 38% V-CM, 17% A-CM	No data	+	No data	No data	U-shape Ca^{2+} wave; rise of calcium in the periphery faster than center	No data	No data
[41]	No data	RyR2 & CSQ	No data	No data	+	No data	No data	++	No data	No data
[35]	No data	No data	54% V-CM, 22% N-CM, 24% A-CM	I_{Ca}, bell shape I-V	No data	No data	No data	No data	No data	No data
[33] [45]	No data	RyR2	No data	I_{Ca}, bell shape I-V	++++	++++	Thapsigargin suppress spontaneous Ca-transients	Suppressed by NCX or RyR2 blockers	Sporadic and brief	2-APB or U73122 slightly affect spontaneous beating
[29]	No data	α-actinin & α-MHC	61% V-CM, 17.4% A-CM and 21.6% N-CM,	No data	No data	No data	No data	Ca^{2+} increase first at periphery then propagate to centre	Stochastic and repetitive	No data
[34]	No data	No data	No data	I_{Ca} bell shape I-V	++++	++++	SERCA Ca^{2+} transport are functional	Slower time to peak and decay rates	No data	No data

CSQ: calsequestrin; PLB Phospholamban; MHC: Myosin heavy chain; RyR2: Ryanodine receptor type 2; NCX: Sodium Calcium exchanger; TPM Tropomyosin

Table 2

Characteristics of 11 hiPSC-CM cell lines expressing CPVT-causing mutations.

CPVT Cell-lines	Content Ca^{2+} store	Ca^{2+} sparks	L-type I_{Ca}	Beating frequency	Diastolic Ca^{2+}	Aberrant Ca^{2+} transients & oscillations	β-Adrenergic responses
Exon3-del[66]	No data	No data	No data			+++++	Beating frequency Diastolic Ca^{2+}
S406L [64]	No change	Bigger and longer	No data	No data	Comparable to control, with Iso	+++++	Spark frequency & decay time
R420Q [44]		No data	No data		with Iso	++ Iso: ++++++	59% insensitive to; 16% arrhythmogenic; 25% $[Ca^{2+}]_i$ rise associated with Ca^{2+} transients
E2311D [65]	No data	No data	No data	No data	No data	+++++ Triggered from multiple foci	DAD frequency
P2328S [43] [66]		No data	No data		Comparable to control	+++++	Diastolic Ca^{2+}
F2483I [27] [33]		Longer & wandering	Comparable to control	with Iso	with Iso	+++++	Beating frequency DAD
T2538R[66]	No data	No data	No data		No change	+++++	Beating frequency
M4109R [67]	No data	No data	No data	No data	No data	+++++	DAD Frequency
L4115F [66]	No data	No data	No data			+++++	Beating frequency
Q4201R [66]	No data	No data	No data	26		+++++	Beating frequency
V4653F [66]	No data	No data	No data			+++++	Beating frequency Diastolic Ca^{2+}